It was revealed the overfilled genome region was 8q24.21. Association with prostate cancer was found with 17 SNPs. 8 SNPs had the intergenic localization: SRRM1P1–CCAT1 (rs16901979, rs12682344, rs6983561, rs10505483) and CASC8–CASC11 (rs10090154, rs4242384, rs7837688, rs4242382); 9 SNPs – intragenic location: PRNCR1 (rs1016343, rs1456315, rs13254738, rs13252298), LOC101930033 (rs445114, rs16902094, rs10505477), CCAT2 (rs6983267), CASC8 (rs1447295).

The early development of the disease was associated with the 9 SNPs: 4 SNPs were located in genes, 5 SNPs had the intergenic localization. It was noted among 9 SNPs 4 markers were in 11q13.3 region (rs7127900, rs7126629, rs7931342, rs11228583). 5 markers (rs6983267, rs10993994, rs7127900, rs7931342, rs17632542) have found in prostate cancer patients and in group of patients with early development.

The 7 SNPs had the association with PSA level (rs16856139, rs12409639, rs10993994, rs3213764, rs1058205, rs1354774, rs2735839). 1 SNP among them was located intergenic region (KLK3–KLK2 rs2735839), 6 SNPs – were in genes (SLC45A3 rs16856139, rs12409639, MSMB rs10993994, ATF7I rs3213764, KLK3 rs1058205, KLKP1 rs1354774). 2 SNPs from 7 (rs10993994, rs2735839) had the relation to PSA level and to prostate cancer patients. Polymorphic variant rs10993994, located near 5′-UTR MSMB gene, had the associations with prostate cancer patients, patients with early start of disease and with PSA level.

In conclusion, the search of genetic markers of prostate cancer development is actual inmodern oncology. These data will allow to form the groups of patients with different outcome and prognosis and perform the personalized therapy for every patient.

http://dx.doi.org/10.1016/j.ejcsup.2015.08.108

A14

Heterogeneity in breast tumor microenvironment: A report from one case

L. Tashireva[a,*], E. Denisov[a,b], O. Savelieva[a,b], M. Zavyalova[a,b,c], E. Kaigorodova[a,b,c], E. Slonimskaya[a], V. Perelmuter[a,c]. [a] *Tomsk Cancer Research Institute, Tomsk, Russian Federation,* [b] *National Research Tomsk State University, Tomsk, Russian Federation,* [c] *Siberian State Medical University, Tomsk, Russian Federation*
[*] Corresponding author.

Background: Intratumoral heterogeneity contributes significantly to the effectiveness of cancer treatment and outcome (Ng C.K., Pemberton H.N., Reis-Filho J.S., 2012). Previously the phenomenon of intratumoral morphological heterogeneity was demonstrated in breast cancer and it was found that the presence of alveolar structures in breast tumors is associated with high frequency of lymphogenic metastasis (Zavyalova M.V. et al., 2013a; Zavyalova M.V. et al. 2013b; Denisov E.V. et al., 2014). Tumor microenvironment (ME) is a key factor in determining the properties of the invasive and metastatic potential of the tumor and seems to be also heterogeneous. Analysis of the ME in patients with different solid tumors reveals that the majority of tumors show a T cell–infiltrated phenotype. Macrophages, fibroblasts, dendric cells etc. are also located in the ME and show their effects. The aim of this work was to determine the expression of key markers of cells which are presented in the ME of the various morphological structures.

Materials and methods: A 49 year-old patient (IC NST, luminal A, T1NxMx, grade 2) was enrolled in this study. The ME of tubular (hollow-like), alveolar (morula-like), trabecular, solid structures/patterns, and discrete (small) groups of tumor cells were isolated from invasive breast carcinoma NST ($n = 1$) using laser microdissection (PALM, Carl Zeiss). RNA was extracted using RNeasy Plus Micro Kit (Qiagen, USA) according to the manufacturer's instructions. cDNA was synthesized, ligated, and amplified using QuantiTect Whole Transcriptome Kit (Qiagen, USA) according to the manufacturer's instructions. The gene expression of ALCAM, Bcl6, CD3E, CD4, CHI3L2, CHID1, FAP, CD244, FoxP3, RORc, CD8, GATA-3, TBX21, EPCAM, CD79, CD206, CD209, IL12, CXCL11, TGFb, IL10 was analyzed using qRT-PCR and normalized to ACTB1 gene and normal tissue.

Results: We showed the prevalence of gene expression in the ME of alveolar and solid structures. At the same time only one gene (GATA-3) was found to be expressed in the ME of trabecular structures and discrete groups of tumor cells. In the ME of alveolar structures we found transcripts involved in Th1-response (CD3E, CD4 and CD8). In addition, expression of GATA-3, TGFb, FAP (fibroblast marker) and CD79 (B-lymphocyte marker) genes linked with the Th2-response was also shown in the ME of alveolar structures. In the ME of solid structures we detected expression of CD3E gene (lymphocyte marker), transcription factors of Th1, Th2 and Th17 cells (TBX21, GATA-3 and RORc genes, respectively), NK cell marker (CD244), fibroblast marker (ALCAM), and IL12 gene.

Conclusion: The obtained data demonstrate heterogeneity of the tumor microenvironment in invasive breast carcinoma NST. Inflammatory (Th1-response) and anti-inflammatory (Th2) reactions were observed both in the ME of alveolar and solid structures. The ME of trabecular structures and discrete groups of cancer cells showed only inflammatory marker. Functional activity of the ME of alveolar structures is determined by expression of TGFb gene, which is involved in anti-inflammatory response, invasion and progression, while in the microenvironment of solid structures IL-12 gene, a key cytokine of Th1 response is expressed.

The study was supported by the Russian Scientific Foundation – Russia (grant No. 14-15-00318) and Russian Foundation for Basic Research – Russia, grant No. 15-34-20864.

http://dx.doi.org/10.1016/j.ejcsup.2015.08.109

P85

Synthesis and anti-cancer effects of CpdA enantiomers, non-steroidal glucocorticoid receptor ligands

L. Tilova[a,*], O. Zadorozhnaya[a], A. Savinkova[a], K. Kirsanov[a], A. Ogloblina[a], G. Belitsky[a], I. Budunova[b], E. Lesovaya[a], M. Yakubovskaya[a]. [a] *Institute of Carcinogenesis, N.N. Blochin Cancer Research Center, Moscow, Russian Federation,* [b] *Northwestern*

University, Feinberg Medical School, Department of Dermatology, Chicago, USA * Corresponding author.

Glucocorticoids (GCs) are the most important component of therapy for a number of diseases including combined chemotherapy of hematological malignancies. However, their application is strongly restricted by the development of serious side effects as well as glucocorticoid resistance. Both side effects and resistance affect child cancer patients more intensively.

Adverse side-effects of glucocorticoid treatment are the result of glucocorticoid receptor (GR)-mediated gene activation, while the beneficial anti-inflammatory effects result from GR-mediated 'transrepression'. Difference in mechanisms of therapeutic and side effects of GCs became obvious and selective glucocorticoid agonists (SEGRA) were developed aiming to separate transactivation from transrepression. Recently we have demonstrated that one of the modern SEGRAs, 2-(4-acetoxyphenyl)-2-chloro-N-methyl-ethylammonium chloride or CpdA, selectively induces transrepression in lymphoma cells and reveals GR-dependent anti-lymphoma activity in vitro and in vivo. However, CpdA is a chiral molecule and exists as a racemic mixture of two enantiomers. Enantiomers often possess the same physical and chemical properties but their biological effects may differ drastically. They differently interact with cell receptors and it can lead to the essential diversity in pharmacokinetics and pharmacodynamics. The notorious example of such a molecule is Thalidomide which was originally designed and used as a sedative drug during the pregnancy, but it was withdrawn chiefly because of its severe teratogenicity. As it was demonstrated, only (R)-thalidomide exhibited significant sedative effects, while (S)-thalidomide revealed the teratogenic effects. The application of enantiopure compounds may lead to reduction of metabolism variability in patients and to decrease of drug effective dose. Thereby, the synthesis of enantiopure isomers of CpdA and the study of their anti-cancer activity are of immediate interest in cancer research.

We synthesized for the first time enantiomers of the CpdA based on literature precedent Sharpless asymmetric dihydroxylation with AD-mix-alpha or AD-mix-beta. (S)- and (R)-enantiomers with enantiopure excess of 98% were obtained. Then we evaluated the cytotoxic effects on acute lymphoblastic leukemia cells CEM and mantle cell lymphoma cells Granta by direct cell counting and found that cytotoxic activities of both enantiomers were comparable with the effect of racemic mixture on cell growth and survival. Glucocorticoids modulate the expression of genes through transrepression and transactivation mechanisms realized in equal measure. In present work we estimated the potential ability of newly synthesized enantiomers to activate these mechanisms.

Transactivation realizes through direct binding of receptor homodimers to specific sequences (glucocorticoid responsive elements (GREs)) in promoter or enhancer regions of GR target genes. Therefore, we studied the level of transactivation as the expression of GR-regulated genes, immunophiline FKBP51 and glucocorticoid-induced leucine zipper GILZ. As shown by Q-RT-PCR, the expression of FKBP51 and GILZ was unaffected after cell treatment by both CpdA enantiomers; hence, these compounds did not induce GR transactivation.

GR-transrepression is chiefly mediated by protein–protein interaction between GR and other transcription factors like NF-kB and AP1, followed by inhibition of their transcriptional activity. We evaluated the transrepression by expression of NF-kB-depended genes, cyclines D1 and D2 (CCND and CCND2). (S)-enantiomer of CpdA down-regulated the expression of CCND1 and CCND2 to the level compared with Dex while (R)-enantiomer surprisingly increased level of CCND1 and CCND2 expression. This fact demonstrated that (S)-CpdA is perspective as antiproliferative and cell growth inhibiting agent.

Overall, our data provide the potential for further studies of CpdA optical isomers, especially (S)-enantiomer, as GR-dependent anti-cancer agents.

http://dx.doi.org/10.1016/j.ejcsup.2015.08.110

A105

Morphological characteristics and clinical significance of tumor infiltrating CD20 B lymphocytes in gastric cancer

O. Tomchuk. *Orenburg State Medical University, Orenburg, Russian Federation*

The expression features of CD20 B-cells in gastric cancer (GC) are not well understood. The aim of the study was to give morphological characteristics of CD20 tumor infiltrating B-lymphocytes (TIL) and evaluate their clinical significance.

We studied the samples of gastric mucosa (GM) adjacent to tumor in 35 patients with gastric cancer who had undergone radical surgery (R0) in Orenburg Regional Clinical Oncology Center between January 2009 and July 2010. The sections were stained immunohistochemically using antibodies to CD20. The density of CD20 B-lymphocytes was calculated using the relative area unit (RAU) equal to $0.42 \times 0.28\ mm^2$ with the magnification×400.

We observed 3 types of structures in which the expression of CD20 B lymphocytes was analyzed: individual cells located diffusely in the GM and the tumor stroma, focal lymphoid infiltrates (LI) and lymphoid follicles (LF). Cells expressing CD20 were of 2 types: round shape with sharp profile and a narrow rim of cytoplasm and irregularly shaped cells with indistinct contours and lots of cytoplasmic processes. There were 8 patients with atypical marked LF germinal center (AGC) arranged around the periphery of the follicle and irregularly shaped. AGC cells had large bright nucleus of round shape with distinct chromatin clumps and did not express CD20.

Correlation between density of CD20 B-lymphocyte in GM and depth of tumor invasion (gamma = −0.474, $p = 0.01$) and the number of lymph node metastases was found (gamma = −0.603, $p = 0.008$). The lowest density value of CD20 B-lymphocytes was observed in three or more metastases (N2) in lymph node (56 + 19.5 + 30.3 + 16.4 and 26.9 + 16.1 cells per unit of area, respectively, with N0, N1 and N2, $p = 0.003$) and depth of invasion G3-4 (55.7 + 19.7 and 32.8 + 19.7 cells per unit of area, respectively, for G1-2 and G3-4, $p = 0.02$).

In turn, the presence of focal LI correlated with the degree of differentiation (gamma = 0.525, $p = 0.02$) and three-year overall survival (gamma = −0.738, $p = 0.03$); the presence of LF correlated with the Grade (gamma = 0.525, $p = 0.02$), histological type (gamma = 0.600, $p = 0.03$) and the three-year relapse-free survival